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## Research report

# The effect of bone morphogenetic protein-7 (BMP-7) on functional recovery, local cerebral glucose utilization and blood flow after transient focal cerebral ischemia in rats

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## Abstract

Bone morphogenetic protein-7 (BMP-7) has been shown to enhance dendritic growth and improve functional recovery after experimental stroke. In this study, we examined the effect of BMP-7 on functional recovery, local cerebral blood flow (LCBF) and local cerebral glucose utilization (LCMRglu) following transient middle cerebral artery occlusion. Sprague–Dawley rats ( $n=29$ ) were anesthetized with halothane/nitrous oxide and received 2-h middle cerebral artery occlusion (MCAo) by poly-L-lysine-coated intraluminal suture. Rectal and cranial temperatures were regulated at 37.0–37.5°C. BMP-7 or vehicle (volume, 25  $\mu$ l) was administered intracisternally in a blinded fashion at 24 h after MCAo. Neurological status was evaluated during occlusion (60 min) and daily for 2 days after MCAo. In matched animal groups, LCMRglu was measured autoradiographically with [ $^{14}$ C]2-deoxyglucose (2-DG) and LCBF with [ $^{14}$ C]iodoantipyrine 48 h after MCAo. Four animals groups were studied: LCMRglu series (BMP-7,  $n=7$ ; vehicle,  $n=8$ ); LCBF series (BMP-7,  $n=6$ ; vehicle,  $n=8$ ). Average three-dimensional image data sets were constructed for each group and were compared by pixel-based statistical methods. Rectal and cranial temperatures, mean blood pressure, plasma glucose and blood gases were similar among groups. BMP-7 significantly improved the total neurological score compared to vehicle at 48 h after MCAo ( $7.3 \pm 0.4$  vs.  $9.0 \pm 0.2$ , respectively;  $P < 0.0003$ ). Compared to vehicle-rats, BMP-7 enhanced glucose utilization in the basal ganglia ipsilateral to stroke and improved LCBF in ipsilateral subthalamus, but decreased LCBF and LCMRglu in contralateral cortical regions. © 2001 Elsevier Science B.V. All rights reserved.

**Theme:** Disorders of the nervous system

**Topic:** Ischemia

**Keywords:** BMP-7; Neuroprotection; Autoradiography; Image-processing; Recovery

## 1. Introduction

Bone morphogenetic protein-7 (BMP-7), also known as osteogenic protein-1 (OP-1), is a member of the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily and has been documented to be a trophic factor for bone and cartilage [38]. Recent studies, however, indicate that the effects of BMP-7 are not limited to the skeletal system. BMP-7 is expressed in fetal kidney, heart, teeth, eye, bone, intestine

and in perinatal neuronal tissues (e.g., hippocampus, cortex and cerebellum) [6,9,24]. BMP-7 selectively promotes *dendritic* growth in cultured sympathetic and CNS neurons — a property that distinguishes it from most other identified growth factors, which largely support axonal outgrowth [37].

Recent reports indicate that BMP-7 exerts neuroprotective effects in the CNS. When administered 24 h after permanent focal cerebral ischemia, BMP-7 led to a significant improvement in the recovery of motor skills without affecting infarction volume [14]. It was suggested that this effect might have been due to its dendritic outgrowth-promoting activity [21,27]. It is possible that BMP-7 may

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have additional mechanisms that promote behavioral recovery after stroke.

We hypothesized that metabolic or hemodynamic factors might contribute to the salutary effect of BMP-7 in focal cerebral ischemia. These factors have not been previously investigated. Thus, the intent of the present study was to examine the effect of BMP-7 on functional recovery, local cerebral blood flow (LCBF) and local cerebral glucose utilization (LCMRglu) in a highly reproducible model of middle cerebral artery occlusion in rats [2].

## 2. Materials and methods

### 2.1. Surgical preparation

Twenty-nine adult male Sprague–Dawley rats (280–335 g, Crl:CD (SD)BR strain, Charles River Laboratories, Wilmington, MA) were used in these studies. The University of Miami's Animal Care and Use Committee approved all study protocols. Animals were fasted overnight but were allowed free access to water. Following atropine sulfate (0.5 mg/kg, i.p.), anesthesia was induced with 3.5% halothane in a mixture of 70% nitrous oxide and a balance of oxygen. Rats were orally intubated, immobilized with pancuronium bromide (0.6 mg/kg, i.v.), and mechanically ventilated. Temperature probes were inserted into the rectum and the left temporalis muscle, and separate heating lamps were used to maintain rectal and cranial temperatures at 37.0–37.5°C (Mon-a-therm 7000; Mallinckrodt, St. Louis, MO). The right femoral artery and vein were catheterized for continuous blood pressure monitoring and periodic blood sampling. Mean arterial blood pressure was measured with a precalibrated Statham pressure transducer (Model P23XL, Viggo-Spectramed, Oxnard, CA) and was recorded continuously (Model RS3400 polygraph; Gould, Valley View, OH). Serial measurements were made of arterial blood gases and pH (Model ABL 330, Radiometer America, Westlake, OH) and plasma glucose (Model 2300 Stat; Yellow Springs Instrument, Yellow Springs, OH). During the 2-day survival period, rectal temperature, and body weight were monitored periodically.

### 2.2. Middle cerebral artery occlusion (MCAo)

MCAo was induced by the intraluminal suture method [40] as modified by us [2]. Under an operating microscope, the right common carotid artery was exposed through a midline neck incision and was carefully dissected free from surrounding nerves and fascia from its bifurcation to the base of the skull. The occipital artery branches of the external carotid artery (ECA) were then isolated, and these branches were dissected and coagulated. The internal carotid artery (ICA) was isolated and carefully separated

from the adjacent vagus nerve, and the pterygopalatine artery was ligated. Next, a 4-cm length of 3-0 monofilament nylon suture was inserted via the proximal ECA into the ICA and thence into the circle of Willis, effectively occluding the middle cerebral artery. In producing MCAo, we made use of a poly-L-lysine-coated suture as previously described [2]. The suture was inserted 19–20 mm from the bifurcation of the CCA, according to the animal's body weight. After the intraluminal suture was placed, the neck incision was closed with a silk suture. Animals were then allowed to awaken from anesthesia and were returned to their cages. Rats that did not demonstrate a left upper extremity paresis during this recovery period were excluded from further study (see Section 2.3). After 2 h of MCA occlusion, rats were reanesthetized with the same anesthetic combination. Temperature probes were reinserted, and the intraluminal suture was carefully removed. The neck incision was closed with silk sutures, and the animals were allowed to survive for 2 days with free access to food and water.

### 2.3. Behavioral testing

Behavioral tests were performed in all 29 rats before MCAo, during occlusion (at 60 min), and at 24 and 48 h after reperfusion. The battery consisted of two tests that have been used previously to evaluate various aspects of neurological function: (1) the postural reflex test to examine upper body posture while the animal is suspended by the tail [1]; and (2) the forelimb placing test to examine sensorimotor integration in forelimb placing responses to visual, tactile and proprioceptive stimuli [5]. Neurological function was graded on a scale of 0–12 (normal score, 0; maximal score, 12), as described by us [2].

### 2.4. Drug administration and experimental groups

BMP-7 was supplied by Creative BioMolecules (Hopkinton, MA), at a concentration of 0.2 mg/ml in 20 mM acetate buffer, pH 4.5, and 5% mannitol. The vehicle solution contained all components except BMP-7 at the same final concentrations and pH.

Twenty-four hours after MCAo, animals were reanesthetized with halothane in 70% N<sub>2</sub>O/30% O<sub>2</sub> for intracisternal injections and were placed in a stereotactic frame. Drug or vehicle was administered intracisternally (volume 25  $\mu$ l) over 2 min in a blinded fashion.

In matched groups, autoradiographic studies of either local cerebral glucose utilization (LCMRglu) or local cerebral blood flow (LCBF) were performed at 48 h after MCAo. Four animal groups were studied: LCMRglu series (BMP-7,  $n=7$ ; vehicle,  $n=8$ ) and LCBF series (BMP-7,  $n=6$ ; vehicle,  $n=8$ ). Rats were randomly assigned to either the LCMRglu or LCBF study groups.

### 2.5. Autoradiographic studies of LCMRglu and LCBF

To measure LCMRglu, the radiotracer [ $^{14}\text{C}$ ]2 deoxyglucose (2-DG) (20  $\mu\text{Ci}$  dissolved in 0.1 ml saline, specific activity 40–45  $\mu\text{Ci}/\text{mmol}$ , New England Nuclear) was administered as an intravenous bolus. Arterial blood samples were taken every 10 s during the first minute, at 1-min intervals for 5 min, and at 2.5–5-min intervals for the remainder of the 45-min study period. Plasma samples were assayed for 2-DG by liquid scintillation counting and for plasma glucose by means of a glucose analyzer (YSI, Yellow Springs, OH).

To measure LCBF, the diffusible radiotracer  $^{14}\text{C}$ -labeled iodoantipyrine (IAP) (20  $\mu\text{Ci}$  dissolved in 1 ml isotonic saline, specific activity 40  $\mu\text{Ci}/\text{mmol}$ , New England Nuclear, Boston, MA) was infused intravenously at a constant rate over 45 s via a Harvard infusion pump. Arterial blood samples were taken at approximately 2-s intervals from a freely flowing femoral arterial line.

Autoradiographic studies were terminated by decapitation. Brains were quickly removed (<1.5 min) and frozen over liquid nitrogen. They were subsequently embedded and sectioned subserially in a cryostat (20- $\mu\text{m}$  thickness, 100- $\mu\text{m}$  intervals) as previously described [42]. These sections, together with calibrated [ $^{14}\text{C}$ ]methylmethacrylate standards, were exposed to Kodak Hyperfilm Beta-max film for 10 days. Films were digitized by means of a Xillix CCD camera (70  $\mu\text{m}/\text{pixel}$  resolution) interfaced to an MCID image analysis system (Imaging Research, St. Catharines, Ontario, Canada). Image files were transferred to a clustered DEC-Alpha minicomputer system (Digital Equipment, Nashua, NH) for processing. Operational equations for the 2-DG method modified for variable plasma glucose [30,34], and for iodoantipyrine method [29], were used to compute LCMRglu and LCBF, respectively.

Averaged autoradiographic image data sets were constructed by means of principles and methods previously published by our group [3,42,43]. Image reconstruction was based on a novel alignment algorithm termed 'disparity analysis', which we have implemented in previous studies (e.g., Refs. [3,41]). In this method, a linear affine transformation was first used to register coronal sections of each brain, and transformation parameters were calculated on the point-to-point basis by disparity analysis. Each coronal section was aligned to its adjacent neighbor by applying a reverse transformation with the estimated translation and rotation parameters. After alignment of LCMRglu or LCBF image data sets in individual rats, corresponding coronal sections of all brains of the LCMRglu series (or the LCBF series, respectively) were placed in register with one another at a common coronal reference level (bregma +0.7 mm). One brain was designated as the template, and all other sections were mapped into its contours at each coronal level by means of an averaging procedure similar to that used for image align-

ment. These procedures resulted in quantitative image data sets depicting average LCMRglu and LCBF for each group, which were displayed in pseudo-color on a video terminal.

Next, a difference image was computed at each level of interest for both the the LCMRglu and LCBF series to compare the BMP-7 and vehicle groups. In order to compare these groups on a pixel-by-pixel basis, we employed one-tailed Mann-Whitney *U*-tests so as separately to define those pixels having significant differences in the direction [BMP-7>vehicle], and those pixels with differences in the opposite direction, i.e. [vehicle>BMP-7]. These statistical maps were displayed in pseudo-color. In addition, region-of-interest (ROI) measurements were performed in standardized atlas-defined regions of neocortex, striatum, thalamus, subthalamus, and hippocampus [44] and were analyzed by repeated-measures analysis of variance (ANOVA).

## 3. Results

### 3.1. Physiological variables

Rectal and cranial (temporalis muscle) temperatures, arterial blood pressure, blood gases, and plasma glucose in the 29 animals studied showed no significant differences among groups when measured before MCAo, during MCAo, and at 15 min, and 24 and 48 h after MCAo. Representative data are shown in Table 1. Although all animals lost weight transiently after surgery, there were no significant differences in body weight between vehicle- and BMP-7-treated rats on days 1 or 2 following ischemia (Table 1).

### 3.2. Neurobehavioral deficits

Contralateral forelimb placing deficits were clearly present at 60 min after MCAo in all rats (Figs. 1 and 2). BMP-7 significantly improved the neurological score compared to vehicle at 48 h after MCA occlusion in both the LCMRglu series and the LCBF series (Fig. 1). Fig. 2 demonstrates significantly improved visual and tactile placing reactions at 48 h in BMP-7-treated rats compared to the vehicle group.

### 3.3. Local cerebral glucose metabolism and blood flow

The quantitative imaging data for LCMRglu and LCBF are presented in Figs. 3–5, and ROI-based measurements from atlas-defined regions are shown in Table 2. Inspection of averaged quantitative autoradiographic images of LCMRglu in the two study groups at nine coronal levels (Fig. 3) revealed striking overall similarities between the two groups at each level (Fig. 3). Inter-group differences were explored by one-tailed Mann-Whitney *U*-tests. The

Table 1  
Physiological variables

	LCMRglu		LCBF	
	Vehicle (n=8)	BMP-7 (n=7)	Vehicle (n=8)	BMP-7 (n=6)
<i>During MCAo (15 min)</i>				
Rectal temp. (°C)	36.8±0.07	36.8±0.08	37.0±0.09	36.8±0.08
Cranial temp. (°C)	36.8±0.04	36.9±0.05	36.9±0.07	36.9±0.03
pH	7.42±0.01	7.42±0.01	7.43±0.01	7.43±0.01
pO <sub>2</sub> (mmHg)	122±4	130±2	116±4	120±3
pCO <sub>2</sub> (mmHg)	38.8±0.5	38.3±0.5	38.4±0.4	38.2±0.4
MABP (mmHg)	99±4	99±3	96±2	101±3
Plasma glucose (mg/dl)	119±4	131±4	126±4	117±6
<i>48 h after MCAo</i>				
Rectal temp. (°C)	36.8±0.06	36.8±0.09	36.8±0.08	36.9±0.06
Cranial temp. (°C)	36.9±0.07	36.7±0.05	36.5±0.05	36.5±0.06
pH	7.48±0.01	7.47±0.01	7.46±0.01	7.44±0.01
pO <sub>2</sub> (mmHg)	119±3	124±6	127±7	127±7
pCO <sub>2</sub> (mmHg)	38.4±0.7	37.1±0.8	37.8±0.8	39.0±0.4
MABP (mmHg)	103±3	104±4	129±3	136±2
Plasma glucose (mg/dl)	208±10	203±9	234±18	203±24
Body weight (g)	267±4	266±7	271±7	256±5

Values are mean±S.E.M.; MCAo, middle cerebral artery occlusion; MABP, mean arterial blood pressure.

only pixels having significant inter-group differences were located at levels 1, 2 or 3; representative difference images and statistical maps are shown in Fig. 4. In the hemisphere *ipsilateral* to MCA occlusion, a coherent zone of significant [BMP-7>Vehicle] LCMRglu difference was present in the basal ganglia. By contrast, regions of significant [Vehicle>BMP-7] LCMRglu difference tended to involve the *contralateral* paramedian and cingulate cortices and patches of lateral cortex (Fig. 4).

Fig. 5 depicts average LCBF image data. For this series, the friable nature of the infarcted tissue led to difficulties in cryostat sectioning and resulted in the production of artifacts in the *ipsilateral* hemisphere, which took the form of anomalous high-flow white streaks. These artifacts were

not present in the *contralateral* hemisphere, which was thus more amenable to reliable data analysis. One-tailed Mann-Whitney *U*-tests revealed that the subthalamic region (the subthalamic nucleus, lateral hypothalamic area and substantia nigra) *ipsilateral* to MCA occlusion contained a coherent zone in which LCBF in the BMP-7 group exceeded that in the Vehicle group (Fig. 5, Table 2). *Contralateral* to MCA occlusion, several cortical and subcortical areas of [Vehicle>BMP-7] LCBF difference were apparent.

#### 4. Discussion

We found that intracisternal injection of BMP-7, administered 24 h after stroke, moderately enhanced the recovery of sensorimotor function following middle cerebral artery occlusion in rats. In addition, BMP-7 significantly increased LCMRglu in *ipsilateral* basal ganglia, improved LCBF in *ipsilateral* subthalamic area, and decreased LCBF and LCMRglu in *contralateral* cortical regions.

Observation of neurological deficits is important not only in clinical stroke patients but also in animal models of cerebral ischemia. The advent of novel medical therapies to protect the ischemic brain has heightened the need to relate the severity and duration of neurological deficits following experimental cerebral ischemia to the characteristics of the antecedent insult [7,39]. It is sometimes difficult, however, to detect neurological deficits after cerebral ischemia in rodents. Previous workers have called attention to a neurological deficit characterized by sen-

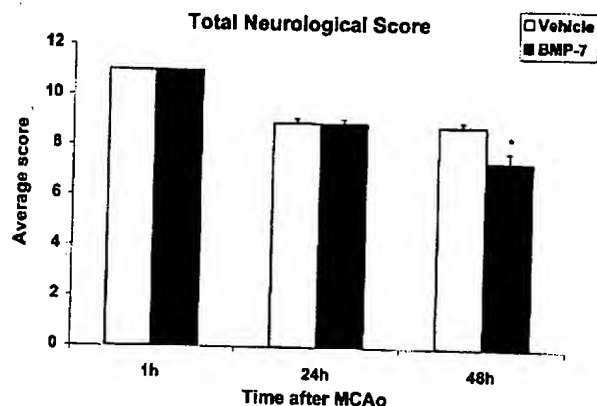


Fig. 1. Total neurological score (normal score, 0; maximal score, 12) at various times after MCAo in BMP-7-treated (n=13) and vehicle-treated rats (n=16). Repeated-measures ANOVA revealed a highly significant treatment effect ( $P=0.002$ ).

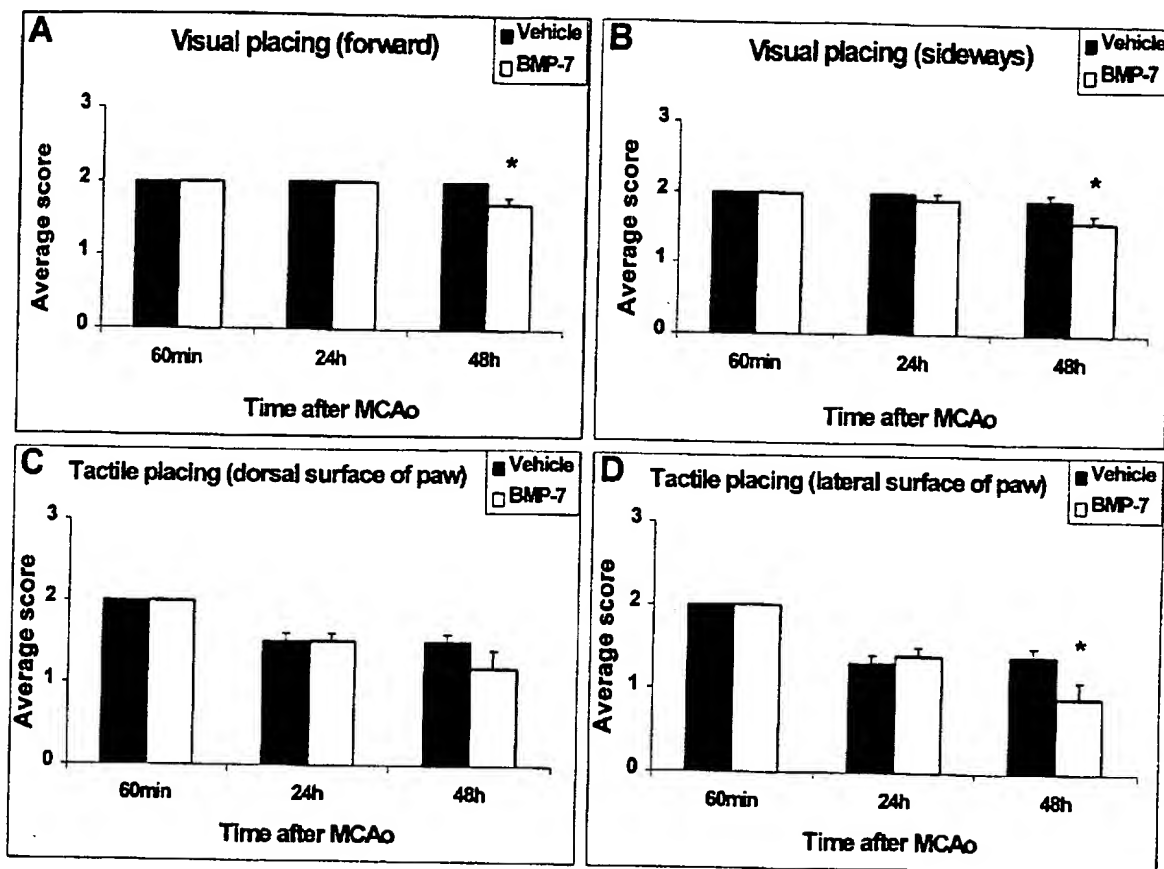


Fig. 2. Time course of recovery of contralateral visual and tactile forelimb placing reactions following MCAo in rats. Bar graphs show improvement of visual (A,B) and tactile placing reactions (D) in BMP-7-treated rats ( $n=13$ ) compared to the vehicle-treated group ( $n=16$ ) (means  $\pm$  S.E.M.). Normal score, 0; maximal score, 2; \* $P<0.05$  (repeated-measures ANOVA).

sensorimotor dysfunction produced by focal ischemia [1,4]. Two tests of the sensorimotor battery appear to be particularly sensitive in detecting deficits following MCAo: the postural reflex test and the forelimb placing test [1,2,5]. The modified model of MCA suture-occlusion used in the present study has the advantage of giving rise to a highly consistent neurobehavioral deficit, with an initially severe disturbance of both visual and tactile placing and a lesser affection of the postural reflex [2,41].

Previous experiments have demonstrated that administration of BMP-7 before transient MCAo [20] or after permanent MCAo [14,27,31] enhances recovery of placing deficit of the contralateral limbs. In the present study, all animals showed normal placing behavior before ischemia, but during MCAo (at 60 min) there were marked contralateral forelimb placing deficits in both groups. Treatment with BMP-7 at 24 h after MCAo significantly improved visual and lateral tactile placing reactions at 48 h compared to vehicle-treated rats. In previous studies, there was no difference in infarct volume, however, between BMP-7- and vehicle-treated rats after permanent MCAo [14,27]. In contrast, pretreatment with BMP-7 24 h before transient

MCAo significantly attenuated the volume of cortical infarction [20]. In previous studies, the recovery of motor functions was first evident 48 h after the administration of BMP-7 and continued for at least 1 month [14].

The mechanism by which BMP-7 enhances functional recovery following stroke in rats is unknown and requires further study. Previous studies in rats have shown that new dendritic and axonal sprouting, together with new synapse formation in both ipsilateral peri-infarct cortex as well as intact contralateral cortex, are likely to be important mechanisms of functional recovery following stroke [11,18,33,37]. Recent studies have indicated that receptors for BMP-7, glial cell line-derived neurotrophic factor (GDNF) and TGF- $\beta$ 1 mRNA are upregulated after brain injury [17,19]. Because BMP-7, GDNF, and TGF- $\beta$ 1 all have neuroprotective effects, the upregulation of these receptors, ligands, or both after cerebral ischemia suggests that endogenous protective mechanisms may be activated after such injury [15,16,36]. Immunostaining for growth-associated protein 43 (GAP-43), a molecular marker of axonal sprouting, showed a selective increase in GAP-43 immunoreactivity in the intact sensorimotor cortex con-

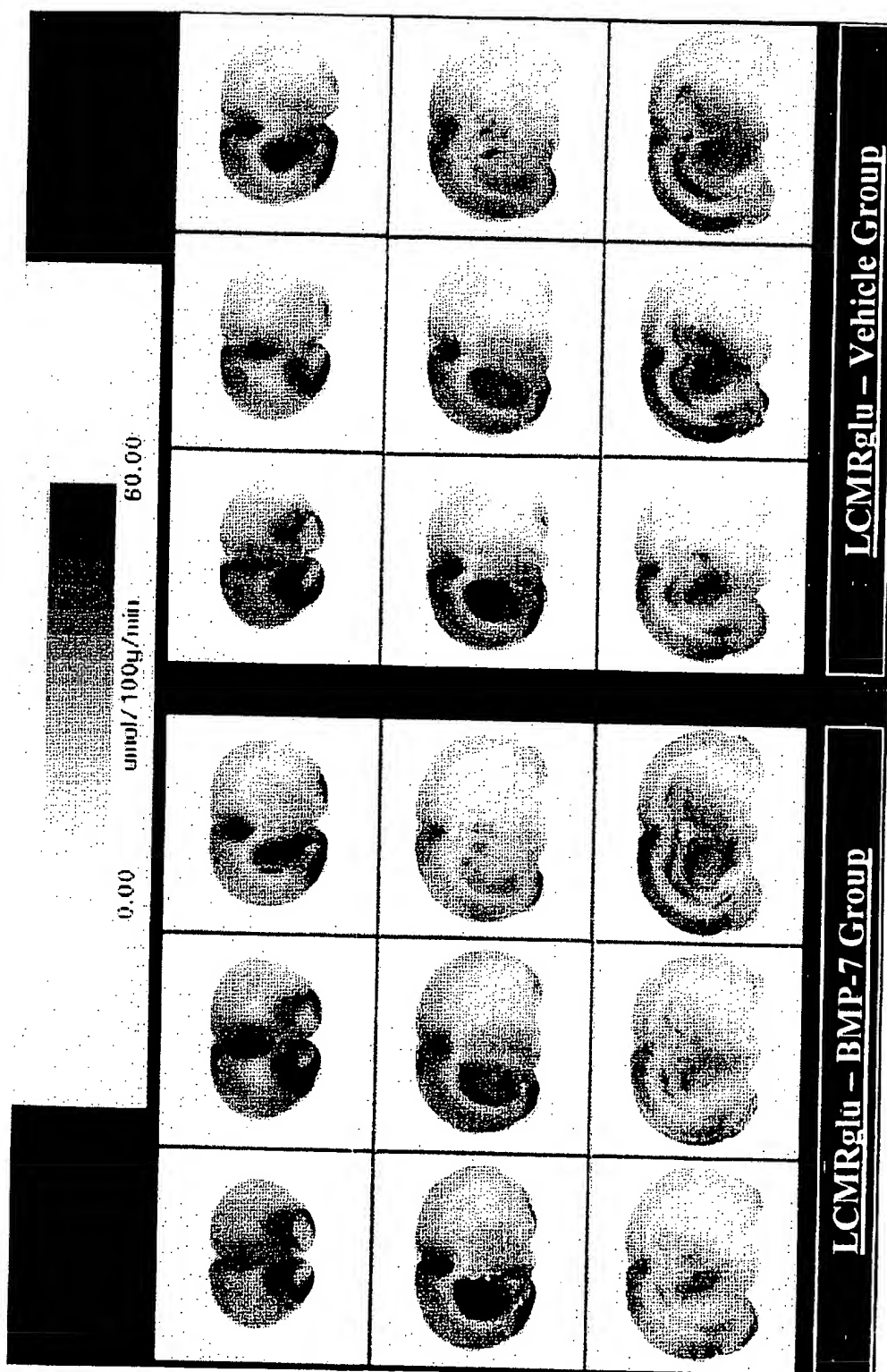


Fig. 3. Local cerebral glucose utilization (LCMRglu) 48 h after MCA occlusion, 24 h after treatment with intracisternal BMP-7 or vehicle. Each image represents a computer-averaged autoradiogram ( $n=7$  in BMP-7 group;  $n=8$  in vehicle group). For each group, nine coronal levels are shown, ranging from bregma level +2.7 to -5.3 mm.

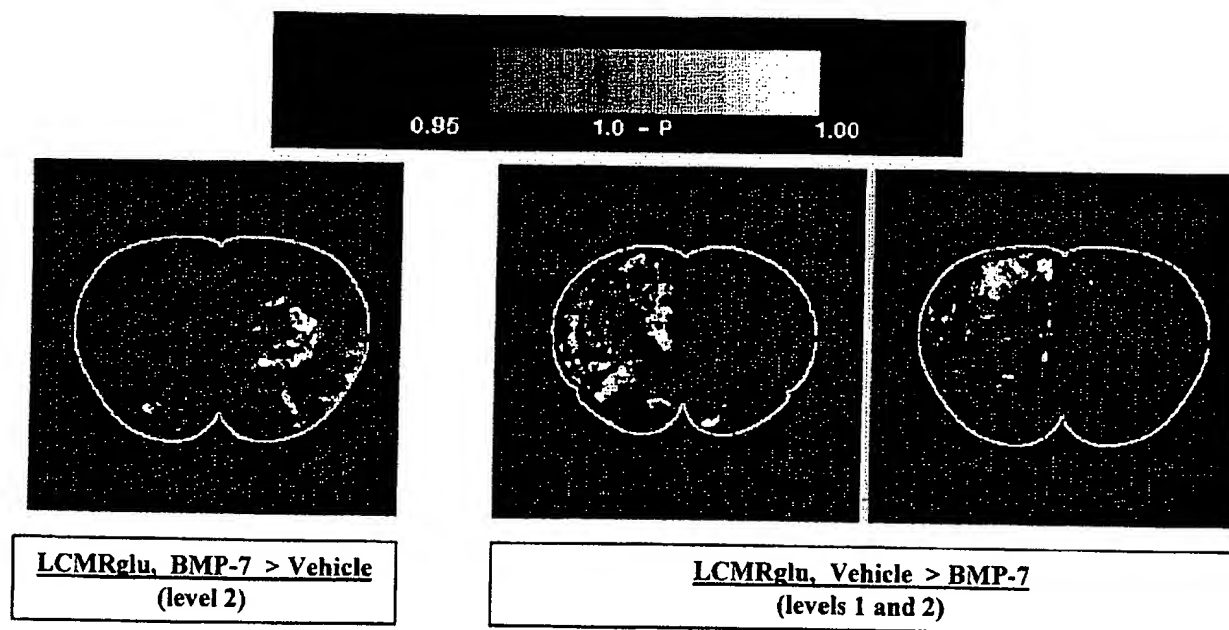


Fig. 4. Statistical maps comparing LCMRglu in BMP-7 and vehicle groups on a pixel-by-pixel basis by one-tailed Mann–Whitney *U*-tests. The gray scale displays  $[1-p]$  at a threshold of 0.95. Thus, visible pixels denote significant differences at the  $P < 0.05$  level. Left panel depicts pixels for which [BMP-7 > vehicle] (at coronal level 2). A zone of significant LCMRglu difference is present in the basal ganglia ipsilateral to prior MCA occlusion. Right panels depict pixels for which [vehicle > BMP-7]. Regions of significant LCMRglu difference involve paramedian and cingulate cortices and patchy zones of lateral neocortex *contralateral* to prior MCA occlusion.

tralateral to cerebral infarct following basic fibroblast growth factor (bFGF) treatment [13].

In addition, it has been reported that BMP-7 stimulates bromodeoxyuridine incorporation into glial cells, resulting in the proliferation of immature glial cells and increasing numbers of astrocytes *in vitro* [12]. Inhibition of bromodeoxyuridine incorporation into the glial cells abolishes BMP-7-induced trophic effects on dopamine neurons. These and other data suggest that BMPs have trophic effects on dopaminergic neurons that are indirectly mediated through activation of glial-derived factors [12].

Similar findings were reported that BMP-7 selectively promotes the differentiation of oligodendroglial-astroglial progenitor cells into astrocytes [22].

Several research groups have been demonstrated that BMPs have a neurotrophic effect on the survival rates of rat dopaminergic neurons in tissue culture [12]. Dopaminergic neurons originating in the substantia nigra have projections primarily to striatum, limbic system, and parts of the neocortex [32]. The highest tissue concentrations of dopamine are found in the striatum and hypothalamus [35]. During MCAo the net efflux of dopamine, in contrast to

Table 2  
Local cerebral glucose utilization and blood flow

	LCMRglu ( $\mu\text{mol}/100 \text{ g}/\text{min}$ )				LCBF ( $\text{ml}/\text{g}/\text{min}$ )			
	Vehicle ( $n=8$ )		BMP-7 ( $n=7$ )		Vehicle ( $n=8$ )		BMP-7 ( $n=6$ )	
	Right	Left	Right	Left	Right	Left	Right	Left
<i>Bregma level +0.7 mm</i>								
Paramedian cortex	26.7 $\pm$ 4.4	41.4 $\pm$ 6.2	26.3 $\pm$ 7.2	37.0 $\pm$ 5.7	1.64 $\pm$ 0.22	1.65 $\pm$ 0.16	1.17 $\pm$ 0.18	1.37 $\pm$ 0.14
Lateral cortex	13.2 $\pm$ 3.8	34.3 $\pm$ 3.8	19.0 $\pm$ 6.8	31.6 $\pm$ 4.0	2.13 $\pm$ 0.28	1.77 $\pm$ 0.18	3.60 $\pm$ 1.23	1.39 $\pm$ 0.18
Striatum	17.0 $\pm$ 3.4	41.2 $\pm$ 5.5	18.2 $\pm$ 4.7	39.3 $\pm$ 6.5	2.36 $\pm$ 0.27	1.44 $\pm$ 0.15	2.37 $\pm$ 0.59	1.06 $\pm$ 0.13
<i>Bregma level -4.3 mm</i>								
Lateral cortex	6.2 $\pm$ 1.5	44.4 $\pm$ 4.7	16.8 $\pm$ 9.8	34.4 $\pm$ 8.0	3.41 $\pm$ 1.37	3.15 $\pm$ 0.89	2.16 $\pm$ 0.27	1.48 $\pm$ 0.17
Central thalamus	20.5 $\pm$ 3.5	42.5 $\pm$ 6.4	19.3 $\pm$ 8.0	31.1 $\pm$ 8.3	1.74 $\pm$ 0.34	1.63 $\pm$ 0.27	1.40 $\pm$ 0.15	1.12 $\pm$ 0.10
Subthalamus	17.2 $\pm$ 3.6	25.0 $\pm$ 5.5	12.4 $\pm$ 6.2	21.9 $\pm$ 6.2	1.24 $\pm$ 0.16	1.10 $\pm$ 0.16	2.26 $\pm$ 0.45*	1.01 $\pm$ 0.12
Dorsal hippocampus	26.4 $\pm$ 3.8	40.4 $\pm$ 4.6	23.2 $\pm$ 7.1	29.6 $\pm$ 6.7	0.96 $\pm$ 0.13	1.04 $\pm$ 0.13	0.63 $\pm$ 0.05	0.69 $\pm$ 0.07

Mean values $\pm$ S.E.M.

\*Different from vehicle, repeated-measures ANOVA,  $P < 0.05$ .



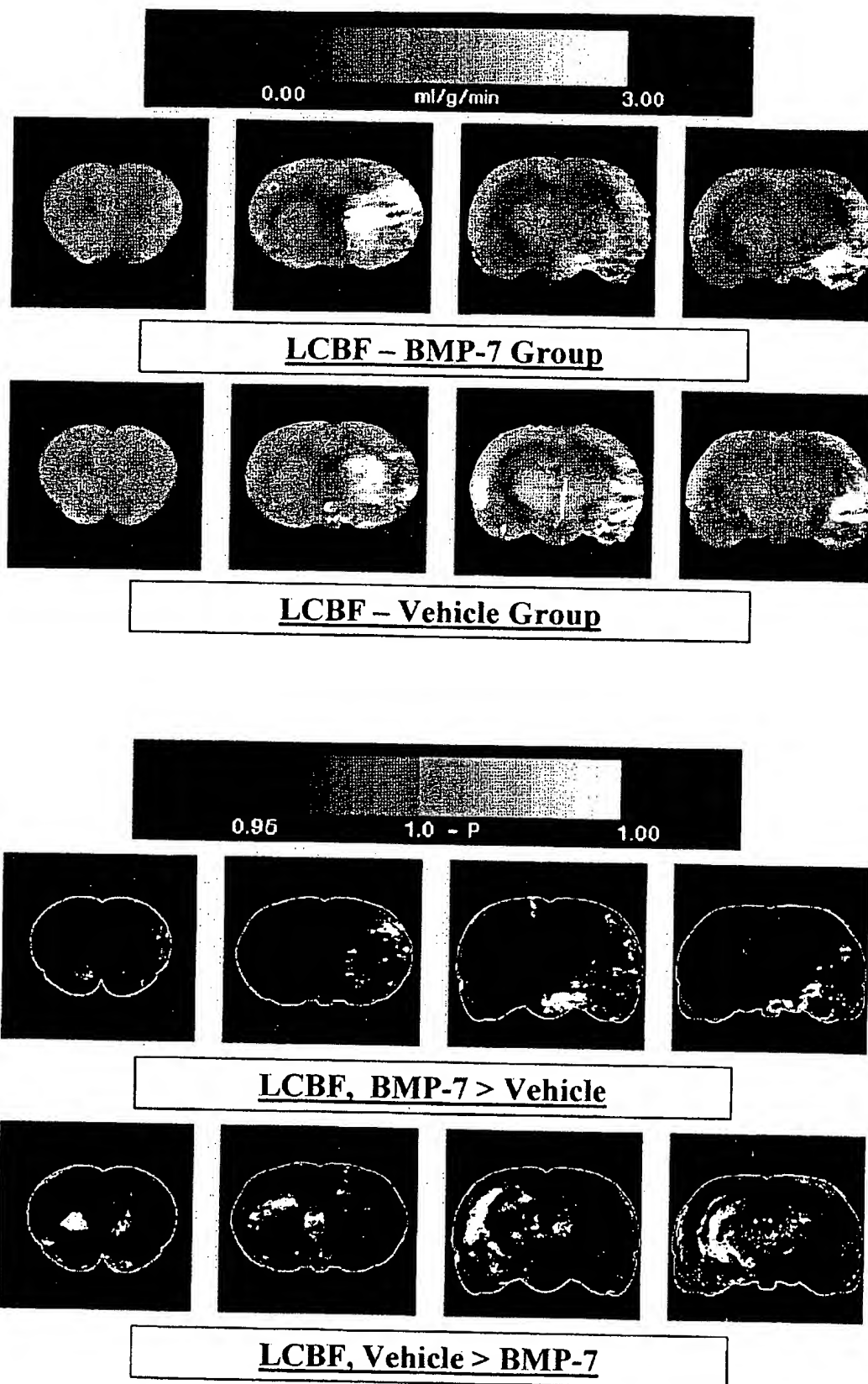


Fig. 5. Top panels: local cerebral blood flow (LCBF) shown at four representative coronal levels (+2.7, +1.2, -4.3 and -5.3 mm with respect to bregma) in animals with prior MCA occlusion treated with either BMP-7 or vehicle. Each image represents a computer-averaged autoradiogram (BMP-7 group,  $n=7$ ; vehicle group,  $n=8$ ). (The white-streaked zones in the hemisphere ipsilateral to prior MCA occlusion are artifacts produced during cryostat-sectioning of these friable, infarcted brains.) Bottom panels: statistical maps of  $[1-p]$  at the same coronal levels as shown in the top-sided panels, based on one-tailed Mann-Whitney  $U$ -tests comparing LCBF in BMP-7- and vehicle-treated groups. Visible pixels denote zones of  $P<0.05$ . Upper four panels depict LCBF zones for which  $[BMP-7 > vehicle]$ . A small region of inter-group LCBF difference involves the subthalamic nuclei, lateral hypothalamic area and substantia nigra. Lower four panels depict LCBF zones for which  $[vehicle > BMP-7]$ . Several such cortical and subcortical regions are apparent in the hemisphere contralateral to prior MCA occlusion.

amino acids, declines [10]. The reason may, at least in part, be related to decreased dopamine levels by inhibition of the oxygen-dependent synthesis of dopamine [23]. Delayed neuronal changes after MCAo were observed in the substantia nigra on the ischemic side [25]. Assays of brain catecholamines revealed 20% reduction of dopamine in the substantia nigra at 5 days after transient MCAo [28]. Nigral lesions also cause a decrease in glutamate efflux and diminished uncoupling of local glucose metabolism and CBF [8]. The reduction of dopamine D-1 receptor sites observed in the substantia nigra of the ischemic side after MCAo is explained by the degeneration of the nerve ending in the strionigral pathway of dopaminergic neurons in the ipsilateral caudate-putamen [25]. In addition, in the suture model of MCAo, cerebral blood flow in the central MCA territory returns to the control level within 3 h of reperfusion, whereas glucose metabolism remains at the same level as during the ischemic period, approximately 40–45% of control [26]. In the present study we found that BMP-7 significantly enhanced glucose utilization in the basal ganglia ipsilateral to stroke and improved LCBF in ipsilateral subthalamic nuclei, lateral hypothalamic area and substantia nigra. We hypothesize that BMP-7 may have rescued dopaminergic cells in this area.

In conclusion, our data indicate that BMP-7 has protective effects in the CNS. Further detailed investigation is required in order to clarify the mechanism of BMP-7. Nonetheless, the current results suggest that BMP-7 may represent a potential new treatment to enhance functional recovery after stroke.

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